

Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1-53. (Canceled)

54. (New) A method of making one or more cDNA molecules, comprising:
- (a) combining one or more RNA molecules with (i) one or more polypeptides having reverse transcriptase activity and (ii) at least one primer adapter nucleic acid molecule, wherein the at least one primer-adapter nucleic acid molecule comprises one or more ligands and one or more cleavage sites, to form a mixture;
 - (b) incubating the mixture under conditions sufficient to make one or more double stranded cDNA molecules, wherein one or more of the cDNA molecules comprise at least one primer-adapter nucleic acid molecule;
 - (c) contacting one or more of the cDNA molecules with at least one hapten to produce one or more hapten-cDNA molecule complexes;
 - (d) cleaving one or more of the complexes with not more than one enzyme, wherein said enzyme cleaves one or more of the complexes at one or more cleavage sites within the primer-adapter, to produce one or more cleaved cDNA molecules; and
 - (e) inserting or ligating one or more of the cleaved cDNA molecules into one or more vectors.
55. (New) The method of claim 54, further comprising isolating one or more of the hapten-cDNA molecule complexes.

56. (New) The method of claim 54, wherein at least one hapten is bound to a solid support.
57. (New) The method of claim 56, wherein the solid support is selected from the group consisting of nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtiter plates.
58. (New) The method of claim 56, wherein the solid support is a magnetic bead.
59. (New) The method of claim 54, wherein the enzyme is *NotI*.
60. (New) The method of claim 54, wherein one or more of the cleaved cDNA molecules comprises one sticky end and one blunt end.
61. (New) The method of claim 60, wherein the sticky end is a *NotI* sticky end and the vector has a *NotI* compatible end and a blunt end.
62. (New) The method of claim 54, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase, an Avian Myeloblastosis Virus (AMV) reverse transcriptase, a Rous Associated Virus (RAV) reverse transcriptase, a Myeloblastosis Associated Virus (MAV) reverse transcriptase, a Human Immunodeficiency Virus (HIV) reverse transcriptase, a retroviral reverse transcriptase, a retrotransposon reverse transcriptase, a hepatitis B virus reverse transcriptase, a cauliflower mosaic virus reverse transcriptase, a bacterial reverse transcriptase, and mutants and variants thereof that are substantially reduced in RNase H activity.

63. (New) The method of claim 54, wherein the conditions sufficient to make one or more cDNA molecules comprise use of one or more DNA polymerases, one or more nucleotides and one or more primers.
64. (New) The method according to claim 63, wherein one or more of the primers are primer-adapters that comprise one or more ligands and one or more cleavage sites.
65. (New) The method of claim 54, wherein at least one of the RNA molecules is an mRNA molecule.
66. (New) The method of claim 54, wherein at least one of the RNA molecules is polyadenylated.
67. (New) The method of claim 54, wherein the one or more RNA molecules is a population of RNA molecules.
68. (New) A method of making one or more cDNA molecules, comprising:
 - (a) combining one or more RNA molecules with (i) one or more polypeptides having reverse transcriptase activity and (ii) at least one primer-adapter nucleic acid molecule, wherein the at least one primer-adapter nucleic acid molecule comprises at least one restriction enzyme recognition sequence and at least one biotin moiety, to form a mixture;
 - (b) incubating the mixture under conditions sufficient to make one or more double stranded cDNA molecules, wherein one or more of the cDNA molecules comprise at least one primer-adapter nucleic acid molecule;
 - (c) contacting one or more of the cDNA molecules with one or more solid supports which comprise avidin and/or streptavidin, to produce one or more solid support-cDNA molecule complexes;

- (d) contacting one or more of the complexes with not more than one restriction enzyme, wherein said restriction enzyme cleaves the at least one restriction enzyme recognition sequence within the adapter-primer, to produce one or more cleaved cDNA molecules; and
 - (e) inserting or ligating one or more of the cleaved cDNA molecules into one or more vectors.
69. (New) The method of claim 68, further comprising isolating one or more of the solid support-cDNA molecule complexes.
70. (New) The method of claim 68, wherein the solid support is selected from the group consisting of nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtiter plates.
71. (New) The method of claim 68, wherein the solid support is a magnetic bead.
72. (New) The method of claim 68, wherein the restriction enzyme is *NotI*.
73. (New) The method of claim 68, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase, an Avian Myeloblastosis Virus (AMV) reverse transcriptase, a Rous Associated Virus (RAV) reverse transcriptase, a Myeloblastosis Associated Virus (MAV) reverse transcriptase, a Human Immunodeficiency Virus (HIV) reverse transcriptase, a retroviral reverse transcriptase, a retrotransposon reverse transcriptase, a hepatitis B virus reverse transcriptase, a cauliflower mosaic virus reverse transcriptase, a bacterial reverse transcriptase, and mutants and variants thereof that are substantially reduced in RNase H activity.

74. (New) The method of claim 68, wherein the conditions sufficient to make one or more cDNA molecules comprise use of one or more DNA polymerases, one or more nucleotides and one or more primers.
75. (New) The method according to claim 68, wherein at least one of the RNA molecules is an mRNA molecule.
76. (New) The method of claim 68, wherein at least one of the RNA molecules is polyadenylated.
77. (New) The method of claim 68, wherein the one or more RNA molecules is a population of RNA molecules.
78. (New) A method of making one or more cDNA molecules, comprising:
 - (a) combining one or more RNA molecules with (i) one or more polypeptides having reverse transcriptase activity and (ii) at least one primer-adaptor nucleic acid molecule, wherein the at least one primer-adaptor nucleic acid molecule comprises one or more ligands and one or more cleavage sites, to form a mixture;
 - (b) incubating the mixture under conditions sufficient to make one or more double stranded cDNA molecules, wherein one or more of the cDNA molecules comprise at least one primer-adaptor nucleic acid molecule;
 - (c) contacting one or more of the cDNA molecules with at least one hapten under conditions sufficient to form one or more hapten-cDNA molecule complexes; and
 - (d) isolating one or more of the complexes comprising the cDNA molecules; and

- (e) cleaving one or more of the isolated complexes with not more than one enzyme, wherein said enzyme cleaves one or more of the complexes at one or more cleavage sites within the primer-adapter.
- 79. (New) The method of claim 78, further comprising inserting or ligating one or more of the cleaved cDNA molecules into one or more vectors.
- 80. (New) The method of claim 78, wherein the hapten is bound to a solid support.
- 81. (New) The method of claim 80, wherein the solid support is selected from the group consisting of nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtiter plates.
- 82. (New) The method of claim 80, wherein the solid support is a magnetic bead.
- 83. (New) The method of claim 78, wherein the enzyme is *NotI*.
- 84. (New) The method of claim 79, wherein one or more of the cleaved cDNA molecules comprise one sticky end and one blunt end.
- 85. (New) The method of claim 84, wherein the sticky end is a *NotI* sticky end and the vector has a *NotI* compatible end and a blunt end.
- 86. (New) The method of claim 78, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase, an Avian Myeloblastosis Virus (AMV) reverse transcriptase, a Rous Associated Virus (RAV) reverse transcriptase, a Myeloblastosis Associated Virus (MAV) reverse transcriptase, a Human Immunodeficiency Virus (HIV) reverse transcriptase, a retroviral reverse transcriptase, a retrotransposon reverse transcriptase, a hepatitis B virus reverse transcriptase, a cauliflower mosaic virus reverse transcriptase, a bacterial reverse transcriptase, and

mutants and variants thereof that are substantially reduced in RNase H activity.

87. (New) The method of claim 78, wherein the conditions sufficient to make one or more cDNA molecules comprise use of one or more DNA polymerases, one or more nucleotides and one or more primers.
88. (New) The method of claim 87, wherein one or more of the primers are primer-adapters that comprise one or more ligands and one or more cleavage sites.
89. (New) The method of claim 78, wherein at least one of the RNA molecules is an mRNA molecule.
90. (New) The method of claim 78, wherein at least one of the RNA molecules is polyadenylated.
91. (New) The method of claim 78, wherein at least one of the RNA molecules is a population of RNA molecules.
92. (New) A method of making one or more cDNA molecules, comprising:
 - (a) combining one or more RNA molecules with (i) one or more polypeptides having reverse transcriptase activity and (ii) at least one primer-adapter nucleic acid molecule, wherein the at least one primer-adapter nucleic acid molecule comprises one or more ligands and one or more cleavage sites, to form a mixture;
 - (b) incubating the mixture under conditions sufficient to make one or more double stranded cDNA molecules, wherein one or more of the cDNA molecules comprise at least one primer-adapter nucleic acid molecule;

- (c) contacting one or more of the cDNA molecules with at least one hapten under conditions sufficient to form one or more hapten-cDNA molecule complexes; and
 - (d) cleaving one or more of the complexes with not more than one enzyme, wherein said enzyme cleaves one or more of the complexes at one or more cleavage sites within the primer-adaptor.
93. (New) The method of claim 92, further comprising isolating one or more of the complexes using a solid support.
94. (New) The method of claim 92, further comprising ligating or inserting the cleaved cDNA molecules into one or more plasmids.
95. (New) The method of claim 92, wherein the hapten is bound to a solid support.
96. (New) The method of claim 95, wherein the solid support is selected from the group consisting of nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtiter plates.
97. (New) The method of claim 95, wherein the solid support is a magnetic bead.
98. (New) The method of claim 92, wherein the enzyme is *NotI*.
99. (New) The method of claim 94, wherein the cleaved cDNA molecules comprise one sticky end and one blunt end.
100. (New) The method of claim 99, wherein the sticky end is a *NotI* sticky end and at least one of the plasmids has a *NotI* compatible end and a blunt end.
101. (New) The method of claim 92, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV)

reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase, an Avian Myeloblastosis Virus (AMV) reverse transcriptase, a Rous Associated Virus (RAV) reverse transcriptase, a Myeloblastosis Associated Virus (MAV) reverse transcriptase, a Human Immunodeficiency Virus (HIV) reverse transcriptase, a retroviral reverse transcriptase, a retrotransposon reverse transcriptase, a hepatitis B virus reverse transcriptase, a cauliflower mosaic virus reverse transcriptase, a bacterial reverse transcriptase, and mutants and variants thereof that are substantially reduced in RNase H activity.

102. (New) The method of claim 92, wherein the conditions sufficient to make one or more cDNA molecules comprise use of one or more DNA polymerases, one or more nucleotides and one or more primers.
103. (New) The method of claim 102, wherein one or more of the primers are primer-adapters that comprise one or more ligands and one or more cleavage sites.
104. (New) The method of claim 92, wherein at least one of the RNA molecules is an mRNA molecule.
105. (New) The method of claim 92, wherein at least one of the RNA molecules is polyadenylated.
106. (New) The method of claim 92, wherein the one or more RNA molecules is a population of RNA molecules.
107. (New) A method of making one or more cDNA molecules, comprising:
 - (a) combining one or more RNA molecules with (i) one or more reverse transcriptases and (ii) one or more primer-adaptor nucleic acid molecules, wherein one or more of the primer-adaptor nucleic acid

molecules comprise at least one restriction enzyme recognition sequence and at least one ligand, to form a mixture;

- (b) incubating the mixture under conditions sufficient to make one or more double stranded cDNA molecules, wherein one or more of the cDNA molecules comprise at least one primer-adaptor nucleic acid molecule;
 - (c) contacting one or more of the cDNA molecules with one or more solid supports which comprise one or more haptens, to produce one or more solid support-cDNA molecule complexes;
 - (d) cleaving one or more of the complexes with not more than one restriction enzyme, wherein said restriction enzyme cleaves the restriction enzyme recognition sequence within the primer-adaptors, to produce one or more cleaved cDNA molecules; and
 - (e) inserting or ligating one or more of the cleaved cDNA molecules into one or more vectors.
108. (New) The method of claim 107, wherein the restriction enzyme is *NotI*.
109. (New) The method of claim 107, wherein the ligand is a biotin moiety.
110. (New) The method of claim 107, wherein the hapten is avidin or streptavidin.
111. (New) The method of claim 54, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase and an Avian Myeloblastosis Virus (AMV) reverse transcriptase.
112. (New) The method of claim 54, wherein the polypeptide is a SUPERScript™ reverse transcriptase.

113. (New) The method of claim 64, wherein the ligand is biotin and the hapten is streptavidin.
114. (New) The method of claim 68, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase and an Avian Myeloblastosis Virus (AMV) reverse transcriptase.
115. (New) The method of claim 68, wherein the polypeptide is a SUPERScript™ reverse transcriptase.
116. (New) The method of claim 78, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase and an Avian Myeloblastosis Virus (AMV) reverse transcriptase.
117. (New) The method of claim 78, wherein the polypeptide is a SUPERScript™ reverse transcriptase.
118. (New) The method of claim 78, wherein the ligand is biotin and the hapten is avidin or streptavidin.
119. (New) The method of claim 92, wherein the one or more polypeptides are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase and an Avian Myeloblastosis Virus (AMV) reverse transcriptase.
120. (New) The method of claim 92, wherein the polypeptide is a SUPERScript™ reverse transcriptase.
121. (New) The method of claim 92, wherein the ligand is biotin and the hapten is avidin or streptavidin.

122. (New) The method of claim 107, wherein the one or more reverse transcriptases are selected from the group consisting of a Moloney Leukemia Virus (M-MLV) reverse transcriptase, a Rous Sarcoma Virus (RSV) reverse transcriptase and an Avian Myeloblastosis Virus (AMV) reverse transcriptase.
123. (New) The method of claim 107, wherein polypeptide is a SUPERScript™ reverse transcriptase.